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suppressive and anti-inflammatory effects as well as proinflammatory and selected immunoenhancing activities. TGF-β appears to stimulate resting cells and to suppress the same cell types if activated. TGF-β when administered systemically acts as an inhibitor, but if given locally can promote inflammation. These bifunctional effects of TGF-β make it difficult to predict its overall contribution to a given inflammatory host reaction.

PDGF AND PDGF RECEPTORS

PDGF and PDGF-like growth factors for mesenchymal cells are basic 30-kD cytokines that are produced by platelet α granules, endothelial cells, fibroblasts, smooth muscle cells, and macrophages (as reviewed in ref. 361). Antibodies to PDGF react with all these products and they all bind to the same receptor for PDGF. PDGF consists of two 17-kD peptide chains ("A" and "B"), with 60% sequence homology, as one of three possible isoforms (AB, AA, or BB). The biological effects of these isoforms is qualitatively similar since they each bind to the same high-affinity cell surface receptor with a K_D of 10^{-9} to 10^{-11} M.

The 180-kD receptor for PDGF is composed of two distinct subunits (α and β) that dimerize upon ligand binding: the A chain of PDGF can bind only to the α subunit, while the B chain of PDGF chain can only bind to the β subunit. Thus the reactivity of a given cell type depends on the total number as well as ratio of α and β receptor subunits (385). Both α and β subunits have single transmembrane regions and their cytoplasmic portions are tyrosine kinases. Part of their signaling mechanism is to activate phospholipase C (by tyrosine phosphorylation), which then induces phosphoinositide turnover and subsequent activation of PKC (386). The PDGF receptors are homologous to the other tyrosine kinase receptors, including M-CSF receptor, c-kit, and the fibroblast growth factor receptor. A wide range of 25,000 to 150,000 receptors are expressed on a variety of cell types including macrophages, neutrophils, fibroblasts, capillary endothelial cells, and smooth muscle cells.

PDGF ACTIVITIES

The wound-healing activities of PDGF are mediated by its mitogenicity for fibroblasts and enhanced production of fibronectin and hyaluronic acid, which are critical components of extracellular matrix and of collagenase, which is vital for scar remodeling (reviewed in ref. 385). PDGF induces IGF-1, which is partly responsible for its mitogenicity (385), and also TGF-β, which initiates collagen production by fibroblasts (387).

PDGF is chemotactic for fibroblasts, monocytes, and neutrophils (385) and this may be mediated by its capacity to induce chemokines, such as MCAF and GRO/MGSA. PDGF is also a potent activator of neutrophil granule enzyme release, superoxide anion production, and enhanced adherence. The wound-healing effects of PDGF are macrophage dependent. Administration of neutralizing anti-PDGF to rats with mesangial proliferative nephritis reduced the mesangial cell proliferation and largely prevented the deposition of extracellular matrix in the kidneys, suggesting PDGF contributes to glomerulonephritis (388). PDGF has been found at sites of inflammation and is involved in the pathogenesis of atherosclerosis (389). This may be abetted by its vasoconstrictive effects and potential for inducing hypoxia. PDGF can enhance immune responses based on its ability to augment the expression of MHC class II antigens on macrophages (390). This may be, in part, attributable to its capacity to induce IFN-γ production by lymphocytes (391). Thus PDGF not only participates in wound repair but also indirectly enhances immune cell-mediated inflammatory reactions.

THE CHEMOKINE FAMILY OF INFLAMMATORY CYTOKINES

Members of the family of chemotactic cytokines (Tables 7 and 8), which have been proposed to be named "chemokines" for short, are being identified as vital initiators and promulgators of inflammatory and immunological reactions (as reviewed in ref. 392). The chemokines range from 8 to 11 kD in MW, are active over a 1- to 100-ng/ml concentration range, and are produced by a wide variety of cell types. They are induced by exogenous irritants and endogenous mediators such as IL-1, TNF, PDGF, and IFN-γ. The chemokines bind to specific cell surface receptors with a K_D of 0.4 to 4 nM. These chemokines can be considered "second-order" cytokines that appear to be less pleiotropic than "first-order" proinflammatory cytokines because they are not potent inducers of other cytokines and exhibit more specialized functions in inflammation and repair. As shown in Table 7, some of the chemokines have been assigned to a "chemokine α" subset based on their gene cluster on chromosome 4 (q12–21) and based on the fact that the first two of their four cysteine groups are separated by one amino acid (C-X-C). This chemokine α group includes IL-8, melanoma growth-stimulating activity (MGSA/GRO), platelet factor 4 (PF-4), β thromboglobulin (βTG), IP-10, and ENA-78. As shown in Table 8, the chemokine β subgroup is located on chromosome 17 (q11–32), has no intervening amino acid between the first two cysteines (C-C), and includes macrophage chemotactic and activating factor (MCAF/MCP-1), RANTES, LD-78 (also known as human MIP-1α, ACT-2, or huMIP-1β), and I-309 (as reviewed in ref. 393).

IL-8 is produced by many cell types including NK

TABLE 7. *Properties of the chemokine α subfamily*

Cytokine	Cell sources	Exogenous stimulants	Endogenous inducers	Chemotactic or haptotactic responses	Major activities
IL-8	Monocytes Neutrophils Fibroblasts Endothelial cells Keratinocytes Large granular lymphs T lymphocytes	Endotoxin Mitogens Particulates Viruses	IL-1 TNF IFN-γ costimulant IL-3	Neutrophils Basophils Unstimulated T cells Melanoma cells	Activates PMN ↑ Neutrophil adhesion ↓ Basophil histamine ↑ Keratinocyte growth Acute inflammation
GRO-αβγ/mu/KC muMIP-2αβ	Monocytes Fibroblasts Endothelial cells	Endotoxin	IL-1 TNF	Neutrophils	Degranulates PMN ↑ Melanoma cell growth ↑ Fibroblast growth Acute inflammation
CTAP III/βTG	Monocytes Platelets	Platelet activators		Fibroblasts	↑ Fibroblast growth
βTG/NAP-2	Monocytes Platelets	Platelet activators		Neutrophils	Activates PMN
PF-4	Platelets	Platelet activators		Fibroblasts	↑ Fibroblast growth Reverses immune suppression ↑ I-CAM1 on E.C.
IP-10/muCRG-2	Monocytes Fibroblasts Endothelial cells Keratinocytes	Endotoxin	IFN-γ	Monocytes Activated T lymphocytes	↑ Chronic inflammation
ENA-78	Epithelial cells		IL-1 TNF	Neutrophils	Activates PMN

cells and T lymphocytes in response to exogenous stimuli such as polyclonal mitogens, injurious stimuli, and infectious agents, as well as proinflammatory cytokines such IL-1 and TNF. IL-8 is a chemoattractant of neutrophils, basophils, and a small proportion (10% or less) of resting $CD4^+$ and $CD8^+$ lymphocytes. IL-8 additionally activates neutrophil enzyme release. IL-8 is also haptotactic for melanocytes and is a comitogenic stimulant of keratinocytes.

IL-8 promotes the adherence of neutrophils to endothelial cells. IL-8 does so by inducing neutrophils to express β_1 integrins. Neutrophils then extravasate by moving between the endothelial cell junctions and through the basement membrane to accumulate in the tissues (394). Intracutaneous injections of IL-8 cause a rapid local neutrophilic infiltration peaking within 3 hr. Intravenous administration of IL-8 does not induce systemic sequelae of elevation of acute-phase proteins or fever but does induce a neutrophilia. Intravenous administration of IL-8 also specifically reduces local peripheral inflammatory responses to IL-8, fMLP, and C5a (395). This transient anti-inflammatory effect of IL-8 probably can be attributed to desensitization of neutrophils by systemically distributed IL-8.

Two distinct but homologous (70% at the amino acid level) receptors for IL-8 have been cloned. The IL-8 receptors are members of the rhodopsin receptor family and have a seven transmembrane spanning region (396,397). The receptors are probably coupled to G-proteins. transduce phosphoinositol hydrolysis, and are capable of rapid elevation of diacylglycerol and cytosolic Ca^{2+} levels, which may lead to activation of protein kinase C (398). IL-8 receptors are expressed by neutrophils, which display both types of IL-8R and their expression is upregulated by G-CSF (A. Lloyd et al., *unpublished results*). Mature neutrophils express about 20,000 receptors per cell. Myelocytic lines and basophils express several thousand receptors per cell.

A murine homologue of human IL-8 has not been identified as yet, but IL-8-like molecules have been identified in rabbits, sheep, and other species. IL-8 is in the circulation of patients with systemic inflammatory reactions or severe trauma. IL-8 has readily been detected in inflammatory sites such as in the synovial fluid in rheu-

TABLE 8. *Properties of the chemokine β subfamily*

Cytokine	Cell source	Exogenous stimulants	Endogenous stimulants	Chemotactic or haptotactic responding cells	Major activities
MCAF/MCP-1/muJE	Monocytes Fibroblasts Endothelial cells Keratinocytes Mesanglal cells	Endotoxin Mitogens Particulates Bacteria	IL-1 TNF PDGF IFN-α	Monocytes Basophils	Macrophage activation Basophil histamine release Chronic inflammation
RANTES	T lymphocytes Platelets	Mitogens Anti-CD3		Monocytes T lymphocytes (memory subset) Eosinophils Basophils	↑ T cell/HUVEC adhesion Basophil histamine release Chronic inflammation
LD-78/muMIP-1α	T lymphocytes Monocytes	Mitogens Anti-CD3 Endotoxin	TNF	Monocytes Activated T lymphocytes (CD8 subset) (eosinophils)	↓ BM progenitor stem cells Costimulates myelopoiesis Activates CD8 lymphocytes ↑ CD8 adhesion to HUVEC
ACT-2/muMIP-1β	T lymphocytes Monocytes	Mitogens Anti-CD3 Endotoxin	TNF IL-2	Monocytes Activated T lymphocytes (CD4 subset)	Costimulates myelopoiesis Activates CD4 lymphocytes ↑ CD4/HUVEC adhesion
I-309/TCA-3	Cultured T lymphocytes	Mitogens		Monocytes	

matoid arthritis (399), extracts of psoriatic skin (400), and in the circulation of patients in septic shock (401). Thus IL-8 is implicated as a major participant in acute as well as more prolonged inflammatory reactions.

MGSA, as its name implies, was first discovered as a factor that accelerated the growth of melanoma cell lines and also as a product of oncogene transfected cell lines (GRO). MGSA/GRO competes for the type II, but not type I, IL-8 receptor on myelocytic cells (402) and is also a potent chemoattractant, as well as activator of neutrophils. MGSA as well as IL-8 has been extracted from psoriatic tissues (403).

GRO has three variants (α, β, and γ), which exhibit about 95% homology in their amino acid sequences. They are probably homologues of murine macrophage derived *KC*, macrophage inflammatory peptides *MIP-2α* and *MIP-2β*. Murine MIP-2α and MIP-2β both compete with equal affinity for type II receptors for IL-8 and chemoattract human as well as murine neutrophils (402). MIP-2 is also reported to degranulate murine neutrophils, resulting in the release of lysosomal enzymes. Local *in vivo* injections of MIP-2 results in neutrophil accumulation and MIP-2 has been isolated from sites of wound healing. MIP-2 is a costimulator of hematopoietic colony formation by CSF-1 and GM-CSF, but the *in vivo* relevance of this observation remains to be established. It is most likely that GRO/MIP-2 inflammatory activities overlap considerably with those of IL-8, and GRO is therefore probably also a major inflammatory mediator.

PF-4 and *CTAP III*, the precursor of βTG, are both present in platelet granules and are released by inducers of platelet aggregation. Consequently, they become available at sites of injury, hemorrhage, and thromboses. Both are reported to chemoattract and to stimulate fibroblasts, presumably for repair purposes. In addition, a 70 amino acid breakdown product of βTG known as neutrophil attracting peptide 2 (*NAP-2*) is a chemoattractant and activator of neutrophils, albeit at 100-fold higher concentrations than IL-8. NAP-2 also competes for the type II IL-8 receptor with about one-hundredth of the affinity of IL-8 (404). However, since at the site of platelet aggregation, high levels of NAP-2 can be released, it is thought to be an active participant in attracting inflammatory cells to such sites.

ENA-78 is the most recently cloned member of the chemokine α subfamily (405). ENA-78 is produced by an epithelial cell line in response to IL-1 and TNF. In cross-desensitization experiments, ENA-78 also utilizes the type II receptor for IL-8 and GRO and is a chemoattractant and activator of neutrophils.

IP-10 is produced by macrophages, endothelial cells, and keratinocytes in response to IFN-γ. The pathophysiological functions of IP-10 remain unclear, but antibod-

ies to IP-10 react with many cell types present at sites of delayed hypersensitivity reactions and IP-10 has been extracted from psoriatic plaques (406). Thus IP-10 can presumably be produced by many cell types and probably participates in chronic inflammation and delayed hypersensitivity responses. A stable recombinant human IP-10 was recently produced by Dr. K. Matsushima (*personal communication*). We have shown that this rhIP-10 is a moderately potent *in vitro* chemoattractant of human monocytes, but not neutrophils. In addition, this IP-10 also is a moderately potent chemoattractant for previously activated CD4 and CD8 T lymphocytes and promotes adhesion of lymphocytes to endothelial cells (D. Taub et al., *unpublished results*). These observations predict that IP-10 will probably be a participant in chronic cell-mediated inflammatory reactions.

Recombinant human and murine members of the chemokine β subfamily have become available for studies only recently. *MCAF*, otherwise known as MCP-1, is produced by monocytes, fibroblasts, and endothelial cells in response to the usual exogenous stimuli, as well as endogenous cytokines such as IL-1, TNF, and PDGF (392,393). MCAF chemoattracts and activates monocytes to release enzymes and become cytostatic for tumor cells. There are no detectable binding sites for MCAF on neutrophils, lymphocytes, or other cell types except monocytes. MCAF regulates the expression of surface adhesion molecules such as integrins, ELAM-1, and CD11c and b on monocytes (407). MCAF has been detected at inflamed atheromatous lesions in blood vessel walls and in the alveolar fluid of patients with pulmonary pathoses. MCAF induces macrophages to accumulate by 6 to 18 hr at sites of injection. Fibrosarcoma tumors that are infiltrated with monocytes have been shown to produce MCAF and to grow more slowly than noninfiltrated tumors. Tumor cells transfected to express MCAF also grow less well *in vivo* than their untransfected counterparts. In addition, MCAF is a potent and rapid degranulator of basophils, resulting in histamine release (408). These data suggest that basophils express MCAF receptors and may play an important role as a late histamine releasing factor (HRF) in the pathogenesis of the late phase of allergic disorders such as atopic food allergies, asthma, and chronic urticaria.

I-309, a product of activated T cells, was recently identified to have chemoattractant activity for monocytes (409). No other biological activities have been reported for I-309 to date.

RANTES is a moderately potent chemoattractant for monocytes and a very potent chemoattractant for memory T cells but not for naive T cells (410). RANTES peptides are produced by activated T lymphocytes and by platelets. Activated T cells respond to a greater extent to RANTES than unstimulated T cells. RANTES has also been detected at sites of atheromatous inflammation. RANTES promotes the adherence of T cells to human vascular endothelial cells (HUVEC) (A. Lloyd et al., *unpublished results*). This response is more marked when anti-CD3 activated T cells as well as IL-1 prestimulated human endothelial cells are used. In addition, RANTES-like MCAF causes rapid basophil degranulation and histamine release and may participate in the late phase of allergic reactions (411).

LD-78 (human MIP-1α), the homologue of murine MIP-1α, has recently been shown to be an *in vitro* chemoattractant for human monocytes and activated T lymphocytes, with a preference for the CD8 subset of T cells (D. Taub et al., *unpublished results*). In addition, huMIP-1α promotes the adhesion of activated CD8 lymphocytes to HUVEC (A. Lloyd et al., *unpublished results*) and also can chemoattract B lymphocytes and eosinophils. ACT-2, which is the human homologue of murine MIP-1β, like MIP-1α chemoattracts monocytes but preferentially attracts activated CD4 rather than CD8 T cells. Similarly, human MIP-1β promotes the adherence of activated CD4 cells to HUVEC. Purified natural MIP-1α and MIP-1β is reported to activate macrophages to be cytotoxic for tumor targets, to secrete TNF, IL-6, and IL-1α, and in the case of mature tissue macrophages to proliferate (412). However, MIP-1α and MIP-1β did not induce an oxidative burst or increased Ia expression by macrophages (412).

Human MIP-1α and MIP-1β show 70% homology in their amino acid sequence. Despite this difference, both MIP-1α and MIP-1β bind with equal affinity to monocytes and T lymphocytes and compete equally well for binding sites on these cell types. However, muMIP-1α is reported to inhibit hematopoietic stem cell replication, while muMIP-1β not only fails to do so but competitively inhibits this activity of MIP-1α (411). Some of the chemokine β ligands utilize the same receptors. Both MIP-1α and MIP-1β compete for about 25% of the MCAF binding sites on monocytes. Conversely, MCAF competes for about 30% of the MIP-1α and MIP-1β binding sites on monocytes, but not for those on lymphocytes (J. M. Wang et al., *unpublished results*). Thus there are shared and unique receptors for the MCAF and MIP-1α chemokines. Although RANTES does not compete for MIP-1α, β or MCAF binding sites, an excess of MIP-1α, MIP-1β, and MCAF undirectionally partially inhibited the RANTES binding sites on macrophage cell lines. These data are supported by experiments showing that the capacity of RANTES to induce calcium influx into these cells can be inhibited by prior incubation with desensitizing doses of MIP-1α and MIP-1β or MCAF, but not the reverse. This suggests that some of the chemokine receptors bind multiple ligands and some of the chemokines bind to multiple receptors.

Overall, the chemokine family members appear to be very potent and pivotal chemoattractants and activators of inflammatory cells and fibroblasts. However, their contribution to immune reactions are still incompletely

defined. The facts that a number of the chemokines are T cell derived, such as RANTES, MIP-1α, β, and I-309, and four of them (RANTES, MIP-1α, β, and IP-10) chemoattract T lymphocytes as well as monocytes suggest that they have a significant immunological role.

CONCLUSION

In conclusion, the aforementioned inflammatory cytokines have myriad effects on cell growth and differentiation. As indicated, some of these effects are indirect and based on the capacity of cytokines to induce the production of a cascade of other cytokines. For example, although TNF and IL-1 exhibit antiviral effects, these are mediated by IFN-β (194). Many reports demonstrate that cytokines not only interact sequentially (one inducing another) but also reinforce one another. For example, TNF not only induces IL-6 and IL-1, but both IL-6 and IL-1 can augment the many biological effects of TNF. Furthermore, IL-1 can even induce IL-1. The interactions become even more complex when one considers the observations that IL-1 and IFN modulate not only the production of other cytokines but can also modulate the expression of functional receptors for cytokines.

All the aforementioned complex *in vitro* interactions of cytokines make it virtually impossible to predict the *in vivo* activities of a given cytokine. This is amply illustrated by the unexpectedly broad spectrum of *in vivo* activities of cytokines such as IL-1, TNF, TGF-β, and IL-6. The actual physiological role of most of the cytokines remains to be established. In fact, we have to relearn the trite but true import of "*in vivo veritas.*" As pure recombinant cytokines of each type become available, it is important to analyze the effects of excess levels of these cytokines on *in vivo* physiological and pathophysiological processes. On the other hand, depleting the cytokines, to determine what vital roles they play, is equally important; this will involve the development of effective reagents (antibodies to cytokines and receptors, soluble receptors, and other specific inhibitors produced by rational drug design) and gene targeting. The cytokines that have been knocked out to date have yielded unexpected results, generally showing less pathological consequences than anticipated.

The advantage of having multiple biochemically distinct cytokines such as IL-1α and IL-1β, TNF and LT, TGF-β1,2,3, and the IFN-α,β family of factors that react, within each family, with the same receptor remains to be established. Despite their apparent similarities, each may serve a unique function, as evidenced by the impact of knocking out just one of the TGF-βs. Perhaps the existence of biochemically different ligands with the same biological activities results in advantageous differences in expression, post-translational processing, *in vivo* half-life, tissue distribution, and access to target cells. The advantage of the apparent redundancy of cytokines, each acting on its own receptor, is also puzzling. For example, IL-1 and TNF and to a lesser extent IL-6 exhibit a broad spectrum of overlapping activities. Perhaps ligands that employ different postreceptor signal transduction pathways when acting together can activate cells at much lower concentrations. This would account for the observed synergistic effects of these cytokines. Of course, the usual reasons for redundancy such as the security of possessing alternative pathways would also be advantagous. Despite the recent explosive progress in cytokine research, there exist a number of gaps, particularly in their intracellular mechanism of action.

Therapeutic usefulness of the cytokines and their inhibitors is growing and should accelerate. We now appreciate the difficulties of systemic administration of the cytokines and it remains a challenge to harness the power nature has invested in these molecules.

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